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Cancer Risk in Women with Hereditary Breast Cancer

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13. ABSTRACT (Maximum 200 Words) Women with breast cancer arising as a consequence of germline mutations in <i>BRCA1</i> or <i>BRCA2</i> are at significant risk of contralateral breast cancer (CBC). Examination of factors influencing contralateral risk will provide insight into prevention strategies for unaffected women with BRCA mutations. A combined analysis performed with the JGH indicates tamoxifen may reduce CBC risk by 53% (attached). To evaluate CBC risk factors, the funded study is assessing the impact of tamoxifen and radiotherapy on CBC risk. In the present funding period, in accordance with the Statement of Work, continued review of clinical records was performed, with identification of pathology blocks that will be cut to provide the samples for genotyping. Medical records from 1990 and 1991 were not reliably retrievable, leading us to expand the cohort eligibility dates to 1996. As of this report, we have reviewed 692 records in this interval, of whom 453 are eligible and for whom data has been entered. Data cleaning is in process and acquisition of tissue samples will begin shortly, followed by genotyping after anonymization. Combining these data with our original cohort of 329 cancers in 305 patients (JNCI 1999), we will have achieved 78% of our accrual goal of 1000 cases.			
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Introduction

Although women with *BRCA1* or *BRCA2* mutations constitute a small minority of breast cancer patients overall, in some populations (e.g. Ashkenazi Jewish women with breast cancer before age 50), an appreciable proportion of cancers are *BRCA*-related. Women with germline mutations are at substantially increased risk of breast cancer, both *de novo* and contralaterally after a first cancer diagnosis. The study of factors influencing the development of contralateral cancer in such women may provide important clues as to the risks and benefits of certain prevention strategies for unaffected individuals. Exposures that promote the development of contralateral disease should likely be avoided by both affected and unaffected heterozygotes, while interventions that reduce contralateral risk are obvious candidates for deployment in the primary prevention arena. Unfortunately, survival and selection biases may confound the study of variables modulating contralateral risk in prevalent groups of women with *BRCA* mutations, who are usually identified through familial cancer clinics. The present study, employing a retrospective anonymized design, circumvents those biases and allows the collection of a relatively unselected group of women with *BRCA* mutations for the examination of factors influencing contralateral risk.

Body

To accomplish the aims of this project, we are extending our previous work examining outcomes in Ashkenazi women with invasive breast cancer. In our original publication, we reported local and systemic outcomes in Ashkenazi women with hereditary breast cancer (associated with *BRCA* founder mutations) treated with lumpectomy and adjuvant radiotherapy between 1980 and 1990. These women were compared to Ashkenazi women without mutations undergoing breast conserving therapy in the same time period. A retrospective, anonymized design was employed to address the difficult human subjects issues relevant to germline predisposition research. We recently combined the data from that study with a similar ascertainment from the Jewish General Hospital in Montreal (Robson ME, Chappuis PO, Satagopan J, Wong N, Boyd J, Goffin JR, Hudis C, Roberge D, Norton L, Bégin LR, Offit K, Foulkes WD. A combined analysis of outcome following breast cancer: differences in survival based on *BRCA1/BRCA2* mutation status and administration of adjuvant treatment. *Breast Cancer Res* 2004, 6:R8-R17, attached as Appendix 1). In that analysis, tamoxifen appeared to reduce the risk of contralateral breast cancer by 53% in Ashkenazi women with

germline mutations undergoing breast conserving treatment for invasive breast cancer. However, because of the relatively small number of mutation carriers, this difference did not reach statistical significance.

Local IRB and Department of Defense HSRRB approval was obtained for extension of the original protocol to allow records review and tissue acquisition from Ashkenazi women receiving treatment (either lumpectomy and radiation, or mastectomy with or without adjuvant radiation) for invasive breast cancer between 1990 and 1995 at Memorial Sloan-Kettering. Final approval to initiate this expansion was received at the end of August 2001, and contracting was completed by 30 October of that year. In accordance with the Statement of Work agreed upon, from October 2001-March 2002, institutional databases were reviewed to identify women diagnosed with invasive breast cancer in that interval from 1990-1992. Hospital registration databases were cross-referenced to identify women of self-reported Jewish religious preference (an effective surrogate for Ashkenazi ancestry in our patient population).

Since March 2002, in accordance with the Statement of Work, clinical data are being gathered by review of medical records, and tissue specimens are being identified through review of pathology databases. At the time of the last Annual Report, we had identified over 800 Jewish women treated for invasive breast cancer at Memorial Sloan-Kettering in 1990, 1991, and the first half of 1992. Of these, 422 had pathology material available, the remainder having received primary surgical therapy elsewhere. Unfortunately, medical records were only retrievable for 25% of these patients, as mentioned in the 2002 Annual Report. The institution converted to an electronic medical record system in 1998. Archived medical records for women who had been inactive patients were not reliably accessible. Because of concerns regarding the possibility of introducing a bias into the ascertainment (women who were survivors were more likely to have active records, which would be retrievable), we moved the ascertainment forward, and concentrated on acquiring records for women diagnosed between 1992 and 1996. Based upon this expansion, we have been able to enter clinical data for 453 additional women diagnosed in this interval, who have pathology material available for genotyping. This data is now being reviewed and cleaned, and we will shortly begin the process of retrieving tissue blocks. We anticipate beginning genotyping in February 2004, completing by April 2004, and completing the data analysis by June 2004. This is slightly behind the schedule outlined in the SOW, due to the extraordinary efforts that we have been exerting to retrieve as many clinical records as possible before beginning the process of anonymization and genotyping.

Once the genotyping is completed, we will have clinical data and genotype data for 782 Ashkenazi women. This will be somewhat less than the 1000 cases projected in the project application, but should be sufficient to identify a significant difference in CBC risk among women taking tamoxifen or not, based upon the experience in our smaller datasets. Whether a difference associated with radiotherapy will be identifiable will be dependent upon the rate of CBC among women undergoing mastectomy along, a variable that has not yet been determined.

Key Research Accomplishments

None as yet

Reportable Outcomes

No outcomes are yet reportable in manuscript form. The results of the impact of tamoxifen on CBC risk in the MSKCC dataset was presented at the 2002 Era of Hope meeting in Baltimore, as projected in the 2002 Annual report. In addition, as described above, an analysis of a combined dataset from MSKCC and Montreal was published this year.

Conclusions

The research is in progress, and thus conclusions would not be appropriate at this time, although preliminary analysis of a subset of the data is supportive of one of the hypotheses of the study, that tamoxifen may reduce the contralateral breast cancer risk in women with *BRCA* mutations who are receiving the drug as adjuvant treatment for their first breast cancer.

References

No directly relevant references have been published since the application, other than the paper attached.

Appendices

1. Reprint of Breast Cancer Research paper

Research article

Open Access

A combined analysis of outcome following breast cancer: differences in survival based on *BRCA1/BRCA2* mutation status and administration of adjuvant treatment

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Abstract

Background: The prognostic significance of germline mutations in *BRCA1* and *BRCA2* in women with breast cancer remains unclear. A combined analysis was performed to address this uncertainty.

Methods: Two retrospective cohorts of Ashkenazi Jewish women undergoing breast-conserving treatment for invasive cancer between 1980 and 1995 ($n=584$) were established. Archived tissue blocks were used as the source of DNA for Ashkenazi Jewish *BRCA1/BRCA2* founder mutation analysis. Paraffin-embedded tissue and follow-up information was available for 505 women.

Results: Genotyping was successful in 496 women, of whom 56 (11.3%) were found to carry a *BRCA1/BRCA2* founder mutation. After a median follow-up period of 116 months, breast cancer specific survival was worse in women with *BRCA1* mutations than in those without (62% at 10 years versus 86%; $P<0.0001$), but not in women with the *BRCA2*

mutation (84% versus 86% at 10 years; $P=0.76$). Germline *BRCA1* mutations were an independent predictor of breast cancer mortality in multivariate analysis (hazard ratio 2.4, 95% confidence interval 1.2–4.8; $P=0.01$). *BRCA1* status predicted breast cancer mortality only among women who did not receive chemotherapy (hazard ratio 4.8, 95% confidence interval 2.0–11.7; $P=0.001$). The risk for metachronous ipsilateral cancer was not greater in women with germline *BRCA1/BRCA2* founder mutations than in those without mutations ($P=0.68$).

Conclusion: *BRCA1* mutations, but not *BRCA2* mutations, are associated with reduced survival in Ashkenazi women undergoing breast-conserving treatment for invasive breast cancer, but the poor prognosis associated with germline *BRCA1* mutations is mitigated by adjuvant chemotherapy. The risk for metachronous ipsilateral disease does not appear to be increased for either *BRCA1* or *BRCA2* mutation carriers, at least up to 10 years of follow up.

Keywords: adjuvant therapy, *BRCA1*, *BRCA2*, breast cancer, contralateral, ipsilateral, prognosis

Introduction

The major breast cancer predisposing genes *BRCA1* and *BRCA2* were identified in 1994 and 1995, respectively [1,2]. Unfortunately, the optimal clinical approach to women who develop hereditary breast cancer remains incompletely defined. Studies of the outcomes of women with *BRCA1/BRCA2*-related cancer have yielded conflicting results. Several reports suggested that women with germline mutations in *BRCA1* are more likely to die from their disease than are women with sporadic breast cancer [3–6]. However, other studies did not confirm these observations [7,8]. Fewer studies have focused on *BRCA2*-related breast cancer [9,10].

Certain unique barriers have impeded the analysis of germline *BRCA1/BRCA2* status as a prognostic factor. Because informed consent is required for genetic testing, and individuals may decline such testing for a variety of reasons, it has been difficult to develop prospective cohorts for outcome studies. On the other hand, testing generally requires the provision of a blood sample, which may introduce a survival bias in the conduct of retrospective studies. Both of these potential selection biases may be avoided through the use of a retrospective design in which *BRCA1/BRCA2* mutations are sought in archival tissue from women who have undergone treatment for cancer, and in whom the clinical outcomes are known. Genetic testing is performed on anonymized tissue specimens, and comparisons are made between the women who do and those who do not have mutations. This design presupposes that the *BRCA1/BRCA2* mutation spectrum in the study group is limited and predefined. This is the case for women of Ashkenazi Jewish descent, because two specific mutations of *BRCA1* (185delAG and 5382insC) and one mutation of *BRCA2* (6174delT) constitute the great majority of mutations identified in this population, and these are easily detectable in paraffin-embedded material [11–14]. The present study employed this retrospective, anonymized design to examine the outcomes of Ashkenazi women with invasive breast cancer undergoing breast-conserving therapy (BCT) at two institutions in the USA and Canada.

Methods

Patients

Patients from Memorial Sloan-Kettering Cancer Center (MSKCC) were identified as previously described [15]. Briefly, clinical databases from the Departments of Pathology, Surgery, and Radiation Therapy were reviewed to identify women who underwent BCT (wide local excision and radiation, with or without axillary node dissection) between 1 January 1980 and 31 December 1990. Hospital registration databases were then cross-referenced to identify women expressing Jewish religious preference. Of the 393 Jewish women undergoing BCT, paraffin-embedded tissue and follow-up information was available for

314 women. Information on tumor grade was not available. The combined impact of germline mutations in *BRCA1/BRCA2* on prognosis in this group of women was reported in a previous publication [15]. For the present study the clinical database was extended and additional analyses were performed evaluating the prognostic impact of interactions between germline status, hormone receptor status, and adjuvant treatment with either tamoxifen or chemotherapy. The clinical impact of germline mutations in *BRCA1* and *BRCA2* were considered separately.

Ashkenazi Jewish women diagnosed with invasive non-metastatic breast cancer at age 65 years or less, who were treated at Sir Mortimer B. Davis-Jewish General Hospital (SMBD-JGH) between 1 January 1980 and 1 November 1995, were identified from the Medical Records Department. Of 292 women identified, 225 underwent breast-conserving surgery. Of these 225 women, 34 did not receive radiation and are excluded from further consideration. Paraffin-embedded tissue and follow-up information was available for the remaining 191 women, who were included in the analysis. Results relating to germline mutations in *BRCA1* for the entire dataset were recently reported [16]. The study was focused on those receiving BCT to harmonize the ascertainment criteria between the two centers and to focus on ipsilateral cancer recurrence. The two studies were combined to increase the sample size and thereby the power to observe the separate effects of mutations in *BRCA1* and *BRCA2*, which was not previously possible with either dataset alone.

Data collection

Study procedures at each institution were approved by the local institutional review board. For each subject, paraffin-embedded material was retrieved from hospital tissue banks and clinical records were reviewed. In order to comply with guidelines regarding the conduct of genetic research on stored tissue samples, only a limited amount of clinical data was extracted from the medical record, including age at diagnosis (<50 or ≥50 years), tumor size (≤2 cm or >2 cm), lymph node involvement (present or absent), hormone receptor status (if available), and adjuvant treatment with chemotherapy or tamoxifen. Time to development of local or distant recurrence was recorded, as was the time to development of contralateral breast cancer, if any. Clinical follow-up information was obtained by chart review, tumor registry query, and contact with the treating physician. The tissue specimen and clinical data for each subject were associated through a unique study identifier. At MSKCC, the link between study identifier and patient identifying information was irretrievably destroyed before genetic analysis was performed. At the SMBD-JGH, the mutation results were anonymized and separated from any personal identifiers in a manner approved by the local institutional review board.

Genetic analysis

Analysis of the paraffin-embedded specimens was performed as previously described [3,15]. Briefly, paraffin sections were obtained from the retrieved tissue blocks and genomic DNA was isolated by standard protocols. At MSKCC normal lymph node was preferentially examined, whereas at SMBD-JGH tumor tissue was studied. All specimens were analyzed by PCR amplification of the regions surrounding the Ashkenazi founder mutations 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*. Radiolabeled PCR products were visualized by denaturing PAGE followed by autoradiography. The detection of characteristic variant bands indicated the presence of the mutation being studied. Variant bands were confirmed by direct sequencing or by an independent PCR amplification of the corresponding DNA sample and repeat analysis. There were no discordant results upon repeat analysis.

DNA samples from nine patients failed to yield PCR products despite repeat analysis. Therefore, the final study group consisted of 496 women. There were 24 women who underwent contralateral BCT for metachronous breast cancer during the period of the study. Thus, for the purposes of determining the rate of metachronous ipsilateral cancer, there were 520 breasts at risk in the dataset.

Statistical analyses

Clinical characteristics of women with and without germline *BRCA1/BRCA2* founder mutations were compared using a χ^2 test. For the survival analyses, breast cancer specific mortality was chosen as the main end-point for the study in order to avoid possible confounding by death due to other causes, such as malignancies of other anatomic sites. Overall survival, defined as the time from diagnosis to death from any cause, was evaluated as a secondary end-point. All ipsilateral breast cancer events were considered in the calculation of the cumulative incidence of metachronous ipsilateral breast cancer, because the retrospective design of this study did not allow reliable discrimination between true recurrences and separate metachronous primaries. All contralateral breast cancer diagnoses were considered new primary lesions for the purposes of calculating the cumulative incidence of contralateral breast cancer. Women who had undergone contralateral mastectomy before the breast cancer diagnosis for which they underwent breast conservation ($n=7$) were excluded from the analysis of this end-point.

Patients were censored if they had not experienced the end-point of interest at the time of last follow up. Kaplan-Meier estimates of breast cancer specific survival time were compared using the log-rank test. A Cox proportional hazards model was used to estimate the hazard ratios (HRs) for breast cancer specific survival time under a multivariate model. All other end-points were analyzed

after accounting for death due to causes other than breast cancer as a competing risk. The cumulative incidences of the end-points in women with and without mutations were compared with a χ^2 test fit using the method of Gray [17]. *P*values comparing the overall curves corresponding to the end-point of interest are provided for these univariate comparisons. The cumulative incidences of death due to breast cancer and due to causes other than breast cancer are both calculated in the same analysis, and shown in separate figures. Multivariate proportional hazards models accounting for non-breast-cancer death as a competing risk were fitted using the method of Fine and Gray [18]. All *P*values were calculated using two-sided tests.

Results

Mutation analysis and clinical characteristics

BRCA1/BRCA2 mutations were identified in 56 (11.3%) of 496 patients (42 *BRCA1* [32 women carried the 185delAG and 10 carried the 5382insC mutation], 13 *BRCA2* [all 6174delT], and one both *BRCA1* and *BRCA2* [185delAG and 6174delT]). The clinical characteristics of women with *BRCA1*, *BRCA2*, and no germline *BRCA1/BRCA2* mutations are presented in Table 1. Women with germline *BRCA1* mutations were significantly more likely than women without these specific mutations to be diagnosed before the age of 50 years, to be diagnosed with estrogen receptor negative tumors, and to have received adjuvant chemotherapy. *BRCA1* mutation carriers were significantly less likely than noncarriers to have received tamoxifen (Table 1). In women with *BRCA1* mutations, neither tumor size nor axillary nodal involvement differed significantly from noncarriers. Among those for whom data were available, *BRCA2* mutation carriers were more likely than *BRCA1* carriers to have estrogen receptor positive tumors (odds ratio 10.5; $P=0.004$) and to have received adjuvant tamoxifen (odds ratio 5.4; $P=0.02$). For no other variable was the distribution significantly different between *BRCA2* mutation carriers and either *BRCA1* carriers or noncarriers.

Breast cancer specific survival

The median follow-up period for surviving women was 116 months. There were 79 women who died from breast cancer, 34 who died from causes other than breast cancer, and one whose cause of death was unknown. There were 18 deaths due to breast cancer among the 57 women with *BRCA1/BRCA2* mutations. Sixteen deaths occurred among 43 *BRCA1* carriers and two among 14 *BRCA2* carriers, and there were 61 breast cancer related deaths among the 439 women who did not have a detectable *BRCA1/BRCA2* mutation. At 10 years, the cumulative incidence of death due to breast cancer among *BRCA1/BRCA2* mutation carriers was 33%, as compared with 14% among noncarriers ($P=0.0002$). However, the negative impact of mutation status was not evenly distributed among *BRCA1/BRCA2* carriers,

Table 1**Clinical characteristics of women with and without *BRCA* founder mutations**

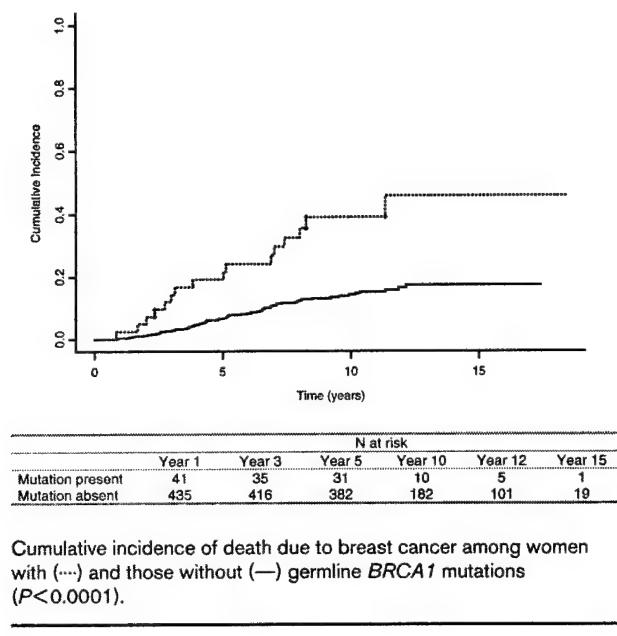
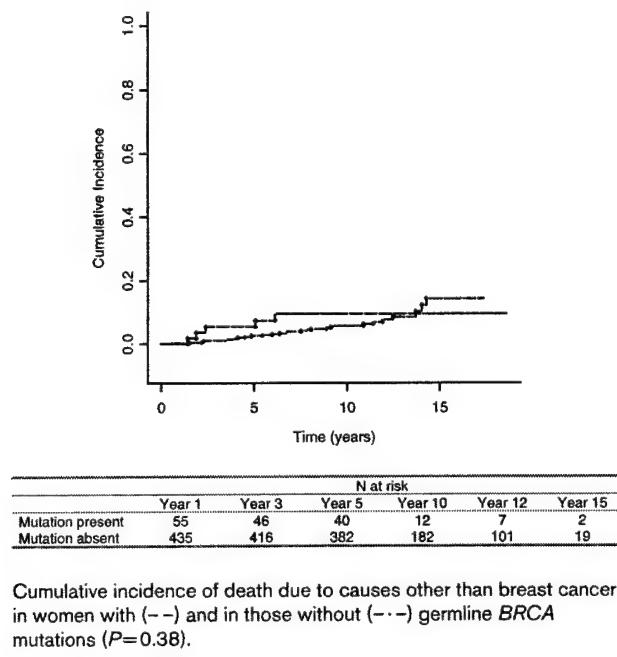
Variable	No <i>BRCA</i> founder mutation (n=440)	<i>BRCA1</i> founder mutation (n=43) [†]	P (<i>BRCA1</i> versus no founder mutation)*	<i>BRCA2</i> founder mutation (n=14) [†]	P (<i>BRCA2</i> versus no founder mutation)*
Age at diagnosis (years)					
< 50	135 (31%)	30 (70%)	< 0.0001	4 (29%)	NS
≥ 50	305 (69%)	13 (30%)		10 (71%)	
Tumor size					
T1	324 (74%)	29 (67%)	NS	8 (57%)	NS
T2	97 (22%)	11 (26%)		4 (29%)	
Unknown	19 (4%)	3 (7%)		2 (14%)	
Nodal involvement					
Present	149 (34%)	18 (42%)	NS	6 (43%)	NS
Absent	262 (60%)	23 (53%)		6 (43%)	
Unknown	29 (6%)	2 (5%)		2 (14%)	
Estrogen receptor					
Positive	197 (45%)	6 (14%)	< 0.0001	7 (50%)	NS
Negative	98 (22%)	27 (63%)		3 (21%)	
Unknown	145 (33%)	10 (23%)		4 (29%)	
Chemotherapy					
Yes	156 (35%)	24 (56%)	0.02	7 (50%)	NS
No	257 (58%)	17 (40%)		7 (50%)	
Unknown	27 (10%)	2 (4%)		0 (0%)	
Tamoxifen					
Yes	190 (43%)	10 (23%)	0.01	9 (64%)	NS
No	203 (46%)	30 (70%)		5 (36%)	
Unknown	47 (11%)	3 (7%)		0 (0%)	

*Cases in which the characteristic of interest is unknown are not included in the comparisons. [†]Woman with both *BRCA1* and *BRCA2* founder mutations are included in both groups, hence the total number women listed here is 497.

because the probability of death due to breast cancer among *BRCA1* mutation carriers was 38% at 10 years ($P<0.0001$, versus noncarriers; Fig. 1 and Table 2) and 15.5% among women with the *BRCA2* founder mutation ($P=0.76$ versus non-carriers; Table 2). There was no difference between women with and those without *BRCA1/BRCA2* mutations with respect to the probability of death due to causes other than breast cancer ($P=0.38$; Fig. 2).

In univariate analyses, age under 50 years at initial diagnosis, presence of a *BRCA1* mutation, primary tumor size greater than 2 cm, estrogen receptor negativity, axillary nodal involvement, and receipt of adjuvant chemotherapy were all significantly associated with poorer breast cancer specific survival (Table 2). Adjuvant treatment was not randomly assigned, and therefore the association between

administration of chemotherapy and a poor outcome ($P<0.0001$) is probably accounted for by the fact that women who were given chemotherapy had a higher *a priori* probability of death than did those not receiving adjuvant chemotherapy. For this reason, use of chemotherapy was not included as a variable in the final multivariate model. In a multivariate competing risk analysis of breast cancer specific survival, we included only those variables that were significant in the univariate analysis. The final, most parsimonious model is shown in Table 3. *BRCA1* mutation status retained independent prognostic significance (HR 2.39, 95% confidence interval [CI] 1.20–4.75; $P=0.01$), along with tumor size greater than 2 cm (HR 2.19, 95% CI 1.30–3.67; $P=0.003$) and axillary nodal involvement (HR 1.78, 95% CI 1.07–2.96; $P=0.03$). Women diagnosed after the age of 50 years were less likely to die from breast cancer than were

Figure 1**Figure 2**

younger women (HR 0.55, 95% CI 0.33–0.92; $P=0.02$; Table 3). The models were essentially unchanged when overall survival was the end-point. The presence of the *BRCA2* mutation was not a significant prognostic factor in either univariate (Table 2) or multivariate analysis (data not shown).

Table 2**Univariate analysis of factors influencing breast cancer specific survival**

Variable	n	Breast cancer deaths (n)	P
<i>BRCA1</i> mutation			
Any	56	18	0.0002
None	439	61	
<i>BRCA2</i> mutation			
Mutation present	43	16	<0.0001
Mutation absent	439	61	
Tumor size			
≤ 2 cm	360	41	<0.0001
> 2 cm	111	31	
Axillary node			
Negative	290	31	<0.0001
Positive	172	44	
Estrogen receptor			
Positive	210	28	0.01
Negative	127	31	
Age at diagnosis (years)			
< 50	169	42	<0.0001
≥ 50	326	37	
Chemotherapy			
Yes	187	48	<0.0001
No	279	30	
Tamoxifen			
Yes	208	28	0.14
No	237	47	

Effect of adjuvant treatment

Of the 468 women in the study for whom information regarding adjuvant chemotherapy was available, 187 received chemotherapy and 281 did not. In univariate analysis the presence of a germline *BRCA1* mutation was significantly associated with a worse breast cancer specific survival both among those women who received chemotherapy (10-year breast cancer mortality 41% in carriers versus 24% in noncarriers; $P=0.04$) and among those who did not (10-year breast cancer mortality 24% in carriers versus 9.6% in noncarriers; $P=0.008$). The presence of a germline *BRCA1* mutation remained an independent predictor of breast cancer mortality in multivariate

Table 3

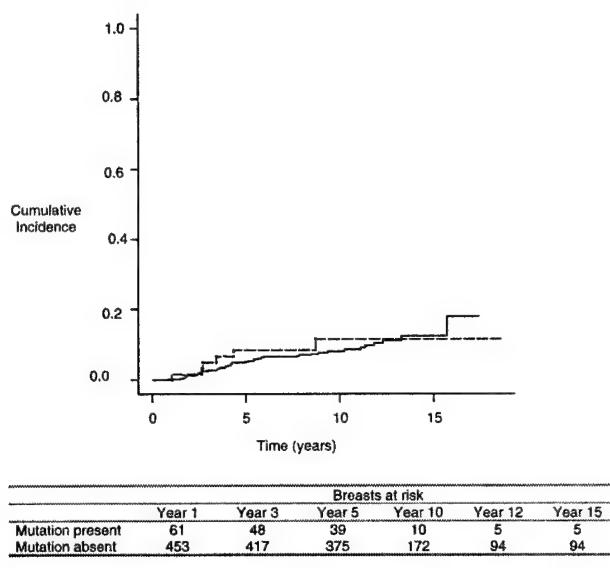
Final multivariate model of breast cancer specific survival				
Variable	n	Deaths due to breast cancer (n)	Hazard ratio (95% CI)	P
<i>BRCA1</i> mutation				
No mutation	397	52	1.00	0.01
<i>BRCA1</i>	37	14	2.39 (1.20–4.75)	
Tumor size				
< 2 cm	333	39	1.00	0.003
≥ 2 cm	101	27	2.19 (1.30–3.67)	
Axillary node				
Negative	280	31	1.00	0.03
Positive	154	35	1.78 (1.07–2.96)	
Age (years)				
< 50	146	34	1.00	0.02
≥ 50	288	32	0.55 (0.33–0.92)	

CI, confidence interval

analysis of the group of women who did not receive chemotherapy (HR 4.8, 95% CI 2.0–11.7; $P=0.001$), but not in those women who did receive adjuvant chemotherapy (HR 1.5, 95% CI 0.66–3.49; $P=0.33$). There were 208 women who received adjuvant tamoxifen and 237 who did not. Tamoxifen usage was not a significant predictor of outcome in univariate analysis. However, in multivariate analysis, among those not receiving tamoxifen, the presence of a germline *BRCA1* mutation was significantly associated with breast cancer specific survival (HR 3.5, 95% CI 1.7–7.2; $P=0.001$). By contrast, *BRCA1* mutations had no prognostic value in those who did receive tamoxifen (HR 0.5, 95% CI 0.05–5.0; $P=0.55$).

Ipsilateral and contralateral breast cancer

A total of 496 patients in the present study underwent BCT for 520 invasive breast cancers. There was no significant difference between women with and those without *BRCA1/BRCA2* mutations in the risk for metachronous ipsilateral breast cancer at 10 years (12% in women with mutations versus 8% in women without; $P=0.68$; Fig. 3). Age under 50 years at initial diagnosis was the only significant predictor of metachronous ipsilateral disease ($P=0.002$). Women with mutations were significantly more likely to develop contralateral breast cancer at 10 years (27% in women with mutations versus 8% in women without; $P<0.0001$, based on a competing cause analysis; Fig. 4). The risk for contralateral cancer was similar in women with *BRCA1* mutations (27% at 10 years) and in those with the *BRCA2* mutation (32% at 10 years). In univariate analyses, which were adjusted for

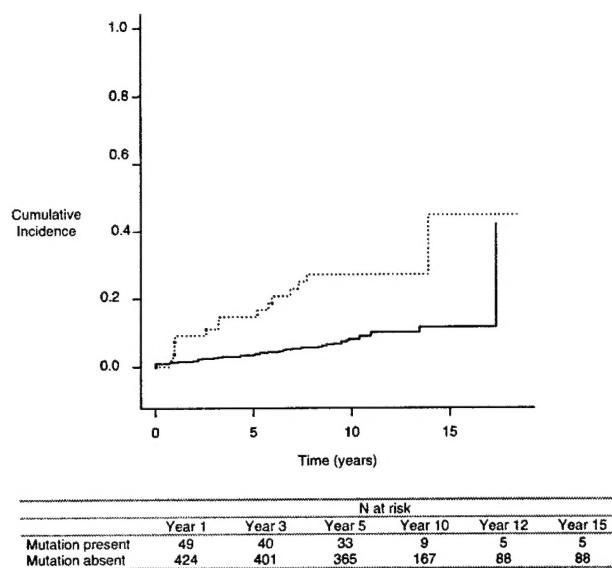
Figure 3Cumulative incidence of metachronous ipsilateral breast cancer in women with (—) and in those without (—) germline *BRCA* mutations ($P=0.68$).

death from competing causes, women not using tamoxifen experienced a higher incidence of ipsilateral breast cancer (HR 1.97, 95% CI 1.01–3.86; $P=0.05$). Those not using tamoxifen also had a marginally, nonsignificantly higher incidence of contralateral breast cancer (HR 1.26, 95% CI 0.71–2.25; $P=0.48$). Chemotherapy did not have a significant effect on either ipsilateral ($P=0.16$) or contralateral ($P=0.36$) breast cancer incidence. In an analysis restricted to mutation carriers, the contralateral breast cancer risk was lower in *BRCA1/BRCA2* mutation carriers receiving tamoxifen than in those not receiving tamoxifen, although this did not reach statistical significance (risk ratio 0.47, 95% CI 0.14–1.59; $P=0.23$). The effect of tamoxifen on contralateral breast cancer risk was not significantly greater in women with a *BRCA2* mutation than in women with *BRCA1* mutations, although this analysis was limited by the small numbers of women in each group.

Discussion

Although *BRCA1* and *BRCA2* were identified in 1994 and 1995, the influence of germline *BRCA1/BRCA2* mutations on the outcome of women with breast cancer has remained undefined. Several retrospective studies of affected mutation carriers identified by familial cancer risk clinics failed to demonstrate any differences in survival when women with mutations were compared with various control groups (for reviews [19,20]). However, because only women who survived their breast cancer were able to undergo genetic testing and be identified as mutation car-

Figure 4



Cumulative incidence of metachronous contralateral breast cancer in women with (---) and in those without (—) germline *BRCA* mutations ($P < 0.0001$).

riers in these prevalent series, a survival bias may have prevented the detection of a negative impact of *BRCA1/BRCA2* mutations on outcome. The anonymized design of the current retrospective study circumvented this potential bias by ensuring that all patients within the study group were genotyped, without regard for vital status.

In the present study, women undergoing BCT for early stage invasive breast cancer demonstrated significantly shorter breast cancer specific and overall survival if they carried a germline *BRCA1* mutation but not a *BRCA2* mutation. This suggests that the clinical outcome of women with germline mutations may vary depending on which gene is mutated, and even possibly on the position of the mutation within a particular gene. However, the present study, examining the consequences of an intentionally restricted set of mutations in a limited sample, cannot definitively resolve this question. Larger studies, preferably of prospectively ascertained incident cohorts, are necessary.

The adverse prognosis conferred by a *BRCA1* mutation was not attributable to differences in tumor size or axillary nodal status, which indicates that the impact of germline status on clinical outcome is not the result of an association with advanced stage at diagnosis. Several studies have demonstrated that breast cancers arising in women with *BRCA1/BRCA2* mutations, particularly those in

BRCA1, frequently manifest adverse pathologic and biologic features, such as high histologic grade, hormone receptor negativity, p53 mutation, aneuploidy, and high S-phase fraction [19]. Studies conducted in sporadic breast cancer have previously demonstrated the negative prognostic significance of each of these factors. Whether the prognostic significance of *BRCA1/BRCA2* status is completely independent of all of these other parameters cannot be resolved by the present study. *BRCA1*-related breast cancers in this series, as in others, were significantly more likely to be estrogen receptor negative than were cancers not associated with *BRCA1* mutations, and estrogen receptor status was associated with breast cancer mortality in univariate analysis. However, germline status remained a significant prognostic indicator in the multivariate model, indicating that the adverse effect of mutation status on outcome is independent of the association with estrogen receptor negative phenotype. The robustness of this observation is limited to some degree by the fact that receptor status was unknown for a significant proportion of patients, in part because some of these cases were diagnosed before routine receptor testing and in part because the small size of some tumors prevented receptor determination using the dextran-coated charcoal method in use at the time.

The design of the present study precluded a separate analysis of the role of grade in determining outcome in *BRCA1* carriers. However, it is known that the majority of *BRCA1*-related breast cancers are high grade, infiltrating ductal breast cancers [21]. It would not significantly change the conclusions of this study if it could be demonstrated that the independent negative impact observed is attributable to the 'downstream' phenotypic consequences of the presence of a *BRCA1* mutation.

Some preclinical studies have demonstrated that mouse embryonic cells lacking functional *Brc1* protein are sensitive to specific chemotherapeutic agents such as cisplatin and mitomycin C [22,23], and similar results have been seen in human breast cancer cells null for *BRCA1* protein [24]. These observations have raised the possibility that human *BRCA1*-associated breast cancers might also be differentially sensitive to chemotherapy. The current analysis is consistent with this hypothesis, because the negative prognostic influence of a germline *BRCA1* mutation appeared to be reduced in women receiving adjuvant chemotherapy, and, in fact, lost independent statistical significance in this group. Furthermore, standard chemotherapy regimens do not include mitomycin-C or cisplatin, and it remains possible that *BRCA1*-associated breast cancers may be even more sensitive to these agents. However, the design of the present study is such that these observations must be considered hypothesis generating. Further exploration should take place within the context of prospective trials.

In the present study, women with germline mutations experienced a risk for metachronous ipsilateral breast cancer that was not significantly different from that in women without mutations. Several studies have described the incidence of subsequent ipsilateral disease in women with *BRCA1/BRCA2* mutations undergoing BCT [7,8,15,25,26]. In these series, ipsilateral tumor recurrence rates ranged from 1% to 20% at 5 years, which are in the range previously reported in series of young women undergoing BCT. A recent analysis of mutation carriers who had survived a mean of 14 years after their breast cancer diagnosis [27] indicated a 49% estimated risk for ipsilateral cancer after 10 years of follow up. However, studies such as this, which analyze prevalent groups of long-term survivors, may overestimate the risk that a newly diagnosed woman with a *BRCA1/BRCA2* mutation will suffer an ipsilateral breast cancer, because a significant fraction of newly diagnosed women will unfortunately die from their breast cancer and thus will not have lived long enough to develop a second ipsilateral tumor. Thus, ipsilateral cancer estimates derived from such studies may be misleading for newly diagnosed women attempting to make the difficult choice between breast conservation and bilateral mastectomy.

Taken together, the results of the available series suggest, at least up to 10 years of follow up, that women with *BRCA1/BRCA2* germline mutations have a risk for ipsilateral recurrence that is similar to that of other young women. Studies based on series of prevalent cases [8,26,27] suggest, however, that women who are long-term survivors may have an increased risk of new second primary malignancies within the treated breast. This interpretation is supported by a further analysis of cases reported by Haffty and colleagues [28], in which all eight patients with deleterious *BRCA1/BRCA2* mutations had new primary ipsilateral breast cancer, whereas patients without deleterious mutations had both true recurrences and new primary breast cancers ($P=0.06$). The cancers occurring in *BRCA1/BRCA2* carriers occurred at a mean time interval of 9.6 years following the initial diagnosis.

Women with *BRCA1/BRCA2* mutations are at substantial increased risk for metachronous contralateral breast cancer throughout the follow-up period. Although this increased contralateral risk could reflect an increased sensitivity to radiation-induced malignancies initiated by scatter from adjuvant radiotherapy, the contralateral incidence rate is similar to that observed in affected mutation carriers after mastectomy [29] and in prospectively followed cohorts of heterozygotes [30,31], which suggests that the observed contralateral breast cancers are most likely the direct result of the underlying genetic predisposition. The apparent paradox of differential ipsilateral and contralateral risk, as well as differences in estimates of ipsilateral risk according to duration of follow up, may be

explained by a model that proposes that women presenting with *BRCA1/BRCA2*-associated breast cancer have multiple foci within both breasts at various stages of evolution toward malignancy. These foci may be eliminated or substantially retarded by adjuvant radiotherapy of the initially affected breast, preventing or delaying their appearance as metachronous ipsilateral cancers. In the untreated contralateral breast, however, occult lesions existing at the time of initial diagnosis could proceed unimpeded in their progression to malignancy, resulting in an unaltered risk for contralateral cancer. Extending the hypothesis, the continued presence of the genetic predisposition may permit the process of carcinogenesis in the affected breast to begin anew once the radiation treatment was completed, and, after a delay, metachronous breast cancers would begin to appear in the treated and untreated breasts at similar rates. This hypothetical model could be tested by careful pathologic examination of breasts of women with *BRCA1/BRCA2* mutations undergoing either therapeutic or prophylactic mastectomy to assess the prevalence of occult premalignant and malignant lesions. The results of early reports are conflicting, but there is some evidence for an excess of premalignant changes, particularly for *BRCA2* mutation carriers [32–34].

Nonsurgical options for breast cancer risk reduction in women with *BRCA1/BRCA2* mutations are limited. A previous case-control study [35] suggested that tamoxifen reduced the risk for contralateral cancer by nearly 50% in mutation carriers, but this benefit was not observed in a prospective trial of primary prevention among unaffected women with *BRCA1* mutations [36]. In the present study, a trend toward a reduction in contralateral breast cancer was seen in mutation carriers who received the drug, although this did not achieve statistical significance. Because the number of women with mutations who received tamoxifen was small, the present series cannot satisfactorily address whether the benefit of tamoxifen in reducing contralateral risk varies between women with *BRCA1* and those with *BRCA2* mutations. It is of interest that the degree of contralateral risk reduction experienced by the mutation carriers taking tamoxifen in the study was similar to that reported in the previous case-control study, and there was no clear difference between the effects observed in *BRCA1* and *BRCA2* mutation carriers, but further studies of larger numbers of women are needed before these data can be used to guide clinical practice.

Retrospective anonymized studies such as the one reported here may be more easily performed in populations with a high prevalence of specific founder *BRCA1/BRCA2* mutations, such as the Ashkenazim. Non-founder mutations are not identified with this design, and a small number of individuals will thus be misclassified as 'mutation negative' when, in fact, they carry a unique dele-

terious alteration. However, the number of such cases is likely to be small. In a recent report of 322 Ashkenazi individuals who underwent full sequence analysis of *BRCA1* and *BRCA2* after negative founder mutation testing, only six (1.9%) were found to carry a unique mutation [37]. Because women undergoing complete sequencing were probably encouraged to do so because of a family history of breast or ovarian cancer, the prevalence of unique mutations is likely to be even lower in an unselected population of breast cancer patients such as those reported in the present study. Thus, false-negative classification is unlikely to have significantly affected the results of this study. Nonetheless, the results of the study may not be applicable to all women with *BRCA1/BRCA2* mutations. The majority of mutation carriers in the study carried either *BRCA1* 185delAG (32 out of 42 [76%] of all *BRCA1* mutation carriers) or *BRCA2* 6174delT. It is possible that other mutant alleles may confer different risks for recurrence.

Conclusion

This combined study demonstrates that women with specific *BRCA1/BRCA2* mutations who develop breast cancer are at increased risk for death from their disease, particularly if the mutation is in *BRCA1*. For up to 10 years after diagnosis, the risk for metachronous ipsilateral cancer is similar to that in women without mutations, but the risk for contralateral disease is substantially higher. Taken together, these findings suggest that the possibility of *BRCA1/BRCA2*-associated breast cancer should be taken into account with other factors when making decisions regarding radiotherapy and adjuvant chemotherapy, but should not be used as a sole factor to determine the appropriateness of BCT. Adjuvant tamoxifen should be given when appropriate to *BRCA1/BRCA2* mutation carriers with hormone receptor positive breast cancer, but despite promising data, the true effectiveness of this agent in reducing contralateral breast cancer risk in this group of women remains uncertain.

Competing interests

None declared

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